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(54) Title: PHARMACEUTICAL PREPARATION

(57) Abstract

This invention relates to a pharmaceutical preparation comprising a combination of one or several receptor/binding proteins (binding growth factors or hormones) and hyaluronic acid (or its derivatives) or a biodegradable polymer, optionally in combination with its/their ligands (growth factors and hormones).

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PHARMACEUTICAL PREPARATION.

The present inv ntion is relating to pharmaceutical preparations consisting of one or several carriers in combination with one or several receptors/binding proteins. The receptors/binding proteins might optionally be connected to suitable ligands, e.g. growth hormone.
By this combination; carrier+receptor/binding protein+e.g. active peptide, according to the invention, a slow release preparation is obtained. Reversely, the principles according to this invention might also be useful in the situation of abnormal, increased production of growth factors, e.g. tumour growth, this time carrier+receptor/binding protein acting as a selective resorption agent of growth factors.

Receptors are protein molecules that can bind hormones and growth factors (=ligands). Each receptor type is specific for its ligand. The receptor function is to convey external e.g. hormonal signals into the target cell. Novel achievements in receptor research have resulted in the possibility to obtain large quantities of specific pure receptor protein. This possibility is fundamental for the present invention which hereby defines a novel therapeutical role of receptors. Some of the known receptors can be exemplified by : Insulin-like growth factor-1-receptor, Insulin-like growth factor-2-receptor, Insulin-receptor, Plateled derived growth factor-receptor, Fibroblast growth factor-receptor, Epidermal growth factor-receptor, Nerve growth factor-receptor, Colony stimulating factor-receptor, Transforming growth factor-receptors, Growth hormone-receptor, Parathyroid hormone-receptor, Calcitonin-receptor, Estrogen-receptor, Tumour necrosis factor-receptor, Insulin-like growth factor serum binding protein and Corticosteroid binding globulin.

It is generally known that growth factors and hormones, both in animals and in humans stimulates important cellular processes concerning cell division, growth, maturation, differentiation. In addition healing and regenerative processes are also regulated by these factors. The growth factors/hormones comprise e.g. Insulin-like growth factor-1 and 2; IGF-1, IGF-2, Platelet derived growth factor; PDGF, Epidermal growth factor; EGF, Fibroblast growth factor; FGF, Nerve growth factor; NGF, Colony stimulating factor; CSF, Transforming growth factor; TGF, Tumour necrosis factor; TNF, Calcitonin; CT, Parathyroid hormone; PTH, Growth

hormone; GH, Estrogens, Bombesin, Bone morphogenetic protein; BMP, Insulin and Corticosteroids.
Insulin, strog ns, corticosteroids, CT and GH are all well known pharmaceutical agents in the daily clinical practice. The research is intense concerning the other different previously mentioned growth factors and hormones. Relating to various studies and results e.g. PDGF and IGF-1 potentiates wound healing, GH increases fracture healing etc. Although most of these growth factors and hormones do have interesting effects according to different recorded data, clinical implications are yet to be found. The results as hitherto obtained indicates that the ways of administration, as presently used, restrict the use of the substances as drugs, e.g. due to the short half-life of peptides, the potency and the potential toxicity of the substances. By the present invention, offering slow release, the bioactivity of the peptides is prolonged by its connection to the carrier materials. The carriers, such as hyaluronic acid, may also have favourable biological effects per se and e.g. a polylactide rod may except being the stabilizing osteosynthetic material also act as a slow release carrier distributing fracture healing promoting polypeptides.

The present invention thus involves (two or) three components; carriers, receptors and ligands. The knowledge of each of these components is extensive (for textbook information see: Ch 12 and 14 in "The molecular biology of the cell", Alberts et al., Garland publ. inc. NY and London; 1989). Based on this information it is clear that a slow release will occur for the peptides described above, if a pharmaceutical preparation is prepared according to the invention.

1. Cross-linking between a matrix gel e.g. hyaluronic acid and a receptor protein can be achieved by a variety of means, e.g. via imidocarbonates, carbonates, oxiranes, aziridines and activated double bonds and halogens. Such strategies have previously been used to immobilize enzymes on polysaccaride gels.
2. By definition a hormone or growth factor-receptor specifically binds its ligand by high affinity and in a reversible manner and the rate of association is much faster than the dissociation rate. These receptor characteristics, high affinity, high specificity and reversibility are thus obligatory in order to use the term receptor. The dissociation rate is usually determined by incubating the receptor with radiolab lled ligands until equilibrium is reached. Excess unlabell d ligand is added and th releas f non-receptorbound radioactivity measured as a function of time reveals the dissociation rate. From th above

stated, it should be clear that the combination of a carrier, with a r ceptor, with a ligand gives, as a general accepted principle, a slow release of th ligand. The present inv ntion introduce receptors in a therapeutical context, in addition it defines carriers allowing slow release.
Below is a schematic practical outline described, revealing the principles to manufacture a pharmaceutical preparation according to this invention.
Example: the preparation of a slow release GH-complex.

a. Isolation of the extracellular domain of the GH receptor is achieved using part of GH receptor cDNA put into an expression vector driven by the MT promotor. Purification of this truncated receptor from media of transfected cells is achieved by affinity chromatography (using hGH Sepharose®) followed by a size separating gel chromatography.
b. Hyaluronic acid is commercially available and is linked to the purified receptor by the use of the crosslinking agent, see above. The high molecular weight complex is purified from remaining GH receptor by repeated centrifugations. Around 75% of crosslinked receptor is functionally intact.
c. The crosslinked hyaluronic acid-GH receptor preparation is then incubated with excess levels of GH for 2 hours at 37 C, unbound hormone is removed by centrifugation.
d. The preparation will, when injected e.g. subcutaneously , slowly release GH in a dose dependent way, not only based upon the amount of GH, but also according to the number of GH-receptors coupled to the gel. The rate of dissociation for a given preparation is determined for each batch by testing the release of immunoreactive GH in vitro (or by testing the release of radioactive GH in animal models).

A. An experiment measuring the increased body weight subsequent to different types of GH-treatment in a group of hypophysectomised (HX) rats was performed. Three HX rats were subcutaneously injected each with a hyaluronic acid-GH-receptor-GH preparation, according to the invention, containing approx 1.2 mg receptor bound GH. Three HX rats subcutaneously injected by the same amount of GH in a water solution served as controls. The slow release treated group had a significant increased bodyweight lasting more than 4 days compared to the controls.

B. A group of rats were injected in the tail by ither 6 mikrograms IGF-1+IGF-1 binding protein+Hyaluronic acid,

or 6 mikrograms IGF-1 in a water solution. Th IGF-1 as used contained trac amounts (200 000 cpm) of radiolabelled IGF-1. Thre rats in ach group were used. After differ nt time points the end of the tail was amputated and radioactivity analyzed. The first group of rats were found to release IGF-1 signifi-cantly slower (more than 2 days) than the controls.

Biodegradable polymers.

Examples of biodegradable polymers are listed below:

Polyglycolide (PGA)
Copolymers of glycolide:
Glycolide/L-lactide copolymers (PGA/PLLA)
Glycolide/trimethylene carbonate copolymers (PGA/TMC)

Polylactides (PLA)
Stereo-copolymers of PLA:
Poly-L-lactide (PLLA)
Poly-DL-lactide (PDLLA)
L-lactide/DL-lactide copolymers
Copolymers of PLA:
Lactide/tetramethylglycolide copolymers
Lactide/trimethylene carbonate copolymers
Lactide / ∂-valerolactone copolymers
Lactide/ $\mathcal{E}$ -caprolactone copolymers
Polydepsipeptides
PLA/polyethylene oxide copolymers
unsymmetrical 3,6-substituted poly-1,4-dioxane-2,5-diones

Poly-ß-hydroxybutyrate (PHBA)
PHBA/ß-hydroxyvalerate copolymers (PHBA/HVA)

Poly-p-dioxanone (PDS)
Poly-∂-valerolactone
Poly- $\mathcal{E}$ -caprolactone
Methylmethacrylate-N-vinyl pyrrolidine copolymers
Polyesteramides
Polyesters of oxalic acid
Polydihydropyran s
Polyalkyl-2-cyanoacrylates
Polyurethanes (PU)

Polyvinylalcohol (PVA)

Polypeptides
Poly-ß-malic acid (PMLA)
Poly-ß-alcanoic acids.

Within the body all of these are degraded by hydrolysis. The different polymers varies in their structural and chemical aspects which gives them different strength, action, degradation-time and usefulness. PDS for instance is used as a resorptive suture-material. The PGA is used in osteosynthetic material as rods, plates and screws e.g. the Biofix®. PLLA ligaments are used, in research, as replacement for the anterior cruciate ligament of the knee etc.
A combination of the different principles, i.e. the stabilizing osteosynthetic material in combination with a local slow release of e.g. fracture-healing promoting peptides, is attractive. The biodegradable polymers structurally being porous and having different grade of coating facilitates the absorption of the growth factor+receptor complex.
C. In an experiment, according the principles above, PGA rods (1.5 x 8 mm) were "loaded" with radiolabelled IGF-1+IGF-1 binding protein (see p. B) using vacuum forces, end parts sealed by melting. The rods were implanted in the end of the tail in three rats. Three control rats were injected in the same part of the tail using the same amount of radiolabelled IGF-1 in a water solution. The slow release rats, according to the invention, had a significant higher radioactivity in tails compared to the controls.

Claims:

1. Pharmaceutical preparation, characterized in that it is a combination of one or several receptor/binding proteins (binding growth factors or hormones) and hyaluronic acid (or its derivatives) or a biodegradable polymer, optionally in combination with its/their ligands (growth factors and hormones).

2. Pharmaceutical preparation, according to claim 1, characterized in that the receptors/binding proteins are mixed with or covalently bound to the hyaluronic acid or the biodegradable polymer.

3. Pharmaceutical preparation, according to claim 1, characterized in that the receptor-binding proteins e.g. are the :
Insulin-like growth factor-1-receptor,
Insulin-like growth factor-2-receptor,
Insulin-receptor,
Plateled derived growth factor-receptor,
Fibroblast growth factor-receptor,
Epidermal growth factor-receptor,.
Nerve growth factor-receptor,
Colony stimulating factor-receptor,
Transforming growth factor-receptors,
Growth hormone-receptor,
Parathyroid hormone-receptor,
Calcitonin-receptor,
Estrogen-receptor,
Tumour necrosis factor-receptor,
Insulin-like growth factor serum binding protein,
Corticosteroid binding globulin.

4. Pharmaceutical preparation, according to claim 1, characterized in that the biodegradable polymers e.g. are:
Polyglycolide (PGA)
Copolymers of glycolide:
Glycolide/L-lactide copolymers (PGA/PLLA)
Glycolide/trimethylene carbonate copolymers (PGA/TMC)

Polylactides (PLA)
Stereo-copolymers of PLA:
Poly-L-lactide (PLLA)
Poly-DL-lactide (PDLLA)
L-lactid /DL-lactide copolymers
Copolym rs of PLA:

Lactid /tetram thylglycolide copolymers
Lactide/trim thyl ne carbonate copolymers
Lactide / ∂-val rolactone copolym rs
Lactide/ Ɛ -caprolactone copolymers
Polydepsipeptides
PLA/polyethylene oxide copolymers
unsymmetrical 3,6-substituted poly-1,4-dioxane-2,5-diones

Poly-ß-hydroxybutyrate (PHBA)
PHBA/ß-hydroxyvalerate copolymers (PHBA/HVA)

Poly-p-dioxanone (PDS)
Poly-∂-valerolactone
Poly- Ɛ -caprolactone
Methylmethacrylate-N-vinyl pyrrolidine copolymers
Polyesteramides
Polyesters of oxalic acid
Polydihydropyranes
Polyalkyl-2-cyanoacrylates
Polyurethanes (PU)

Polyvinylalcohol (PVA)

Polypeptides
Poly-ß-malic acid (PMLA)
Poly-ß-alcanoic acids

5. Pharmaceutical preparation, according to claim 2, characterized in that the ligands e.g. are :
Insulin-like growth factor-1 and 2; IGF-1, IGF-2,
Platelet derived growth factor; PDGF,
Epidermal growth factor; EGF,
Fibroblast growth factor; FGF,
Nerve growth factor; NGF,
Colony stimulating factor; CSF,
Transforming growth factor; TGF,
Tumour necrosis factor; TNF,
Calcitonin; CT,
Parathyroid hormone; PTH,
Growth hormone; GH,
Estrogens,
Bombesin,
Bone morphogen tic prot in; BMP,
Corticost r ids,
Insulin.

AMENDED CLAIMS

[received by the International Bureau on 29 March 1990 (29.03.90) original claims 1 and 3 amended ; other claims unchanged (3 pages)]

1. Pharmaceutical preparation for use in vivo, characterized in that it is a combination of one or several receptor/binding proteins for binding growth factors or hormones and hyaluronic acid, or its derivatives, or a biodegradable polymer; optionally in combination, with its/their ligands to achieve slow release of growth factors and hormones.

2. Pharmaceutical preparation, according to claim 1, characterized in that the receptors/binding proteins are mixed with or covalently bound to the hyaluronic acid or the biodegradable polymer.

3. Pharmaceutical preparation, according to claim 1, characterized in that optionally modified, such as truncated, receptors/binding proteins e.g. are:
Insulin-like growth factor-1-receptor,
Insulin-like growth factor-2-receptor,
Insulin-receptor,
Platelet derived growth factor-receptor,
Fibroblast growth factor-receptor,
Epidermal growth factor-receptor,.
Nerve growth factor-receptor,
Colony stimulating factor-receptor,
Transforming growth factor-receptors,
Growth hormone-receptor,
Parathyroid hormone-receptor,
Calcitonin-receptor,
Estrogen-receptor,
Tumour necrosis factor-receptor,
Insulin-like growth factor serum binding protein,
Corticosteroid binding globulin.

4. Pharmaceutical preparation, according to claim 1, characterized in that the biodegradable polymers .g. are:
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Polylactides (PLA)
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Poly-DL-lactide (PDLLA)
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Copolymers of PLA:
Lactide/tetramethylglycolide copolymers
Lactide/trimethylene carbonate copolymers
Lactide / ∂-valerolactone copolymers
Lactide/ ε-caprolactone copolymers
Polydepsipeptides
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Poly-ß-hydroxybutyrate (PHBA)
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Poly-p-dioxanone (PDS)
Poly- ∂-valerolactone
Poly- ε-caprolactone
Methylmethacrylate-N-vinyl pyrrolidine copolymers
Polyesteramides
Polyesters of oxalic acid
Polydihydropyranes
Polyalkyl-2-cyanoacrylates
Polyurethanes (PU)

Polyvinylalcohol (PVA)

Polypeptides
Poly-ß-malic acid (PMLA)
Poly-ß-alcanoic acids

5. Pharmaceutical preparation, according to claim 2, characterized in that the ligands e.g. are :
Insulin-like growth factor-1 and 2; IGF-1, IGF-2,
Platelet derived growth factor; PDGF,
Epidermal growth factor; EGF,
Fibroblast growth factor; FGF,
Nerve growth factor; NGF,
Colony stimulating factor; CSF,
Transforming growth fact r; TGF,
Tumour necrosis factor; TNF,
Calcitonin; CT,
Parathyroid hormone; PTH,

Growth hormone; GH,
Estrogens,
Bombesin,
Bone morphogenetic protein; BMP,
Corticosteroids,
Insulin.

INTERNATI NAL SEARCH REPORT

International Application No PCT/SE 89/00666

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 9/22, 47/00, 37/02, 37/36//A 61 L 27/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	A 61 K; A 61 L; C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	WO, A1, 88/09818 (GENENTECH INC.) 15 December 1988, see page 17 lines 26-35; example 8; claim 31 --	1-3,5
X	US, A, 4687808 (R. D. JARRETT ET AL.) 18 August 1987, see claims 1-5; column 5 lines 25-61 --	1,2,4
X	US, A, 4469681 (M. BROWNLEE ET AL.) 4 September 1984, see column 5 line 10 - column 6 line 2; column 6 line 61 - column 7 line 10; column 8 lines 7-43 --	1,2

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th January 1990	1990 -01- 29
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Niklas Forslund

III. DOCUMENTS C NSIDERED T BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A1, 89/04838 (IMMUNEX CORPORATION) 1 June 1989, see especially claim 23 --	1,2
P,X	US, A, 4828563 (W.G.K. MÜLLER-LIERHEIM) 9 May 1989, see the whole document --	1-5
X	EP, A2, 0103184 (DIAMOND SHAMROCK CORPORATION) 21 March 1984, see page 9 and claims --	1,2,4
X	EP, A2, 0014995 (PHARMACIA AB) 3 September 1980, see the whole document --	1
X	WO, A1, 88/07078 (DR. MÜLLER-LIERHEIM AG) 22 September 1988, see the whole document	1,2,4

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 89/00666

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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